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DOCUMENT NO. _____
NO CHANGE IN CLASS ☒
DECLASSIFIED
CLASS. CHANGED TO: TS S C
NEXT REVIEW DATE: _____
AUTH: HR 70-2
DATE *8/6/82* REVIEWER: *03/169*

DOCUMENTS BRANCH
TRANSLATION

Number 63

23 Apr 1947

F. B. C. G. 77

49

Prepared By
Documents Branch
CENTRAL INTELLIGENCE GROUP
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CHEMICAL SEPARATION AND DETERMINATION OF FATTY ACIDS

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CHEMICAL SEPARATION AND DETERMINATION OF FATTY ACIDS

PART ONE: SATURATED HYDROXAMIC ACIDS
PART TWO: UNSATURATED HYDROXAMIC ACIDS

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(KYOTO Imperial University Agricultural Department Organic Chemistry Laboratory)

Received : 9 May 1940

COUNTRY: JAPAN
SUBJECT: FATTY ACIDS
ORIGIN: JAPANESE DOCUMENT

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SATURATED HYDROXAMIC ACIDS:

Concerning methods of separating and determining the individual fatty acids of a mixture of fatty acids obtained from natural oils and fats, there have been since the earliest times of chemical interests in fats many studies and proposals made; however there are still faults to be found in the present methods and suggestions and no ideal method can be discovered.

In order to separate the highly-unsaturated acids found in the unusually rich supply of fish oils in Japan and to determine the chemical composition of these oils, it is especially imperative that a method of purifying and isolating them be found.

As long as even a single acid cannot be isolated as a simple crystalline derivative then complete reliance cannot be placed on the present widely employed separation methods which depend upon bromidization and distillation at decreased pressures and fixed temperatures.

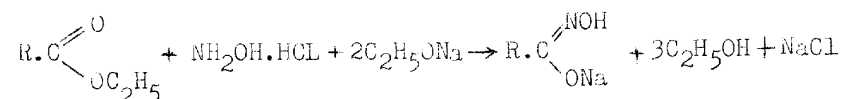
With the object of contributing even a little to this field of inquiry the authors caused hydroxylamine to react on fatty acids; because the hydroxamic acids which they obtained thus are crystalline substances they made use of this discovery and proceeded to carry out basic experiments on these materials.

In order to obtain hydroxamic acids one may use the method of deriving it from acid anhydrides or acid chlorides, amides, and esters of fatty acids; the authors however experimented chiefly on esters or glycerines in order to prepare it directly from fatty acids.

The reasons that we thought it advantageous to separate and determine chemically fatty acids in the form of hydroxamic acids are as follows:

- 1- They can be obtained as crystals of high melting point from any fatty acids.
- 2- They easily combine with metals to form salts and therefore they can be prepared in pure form.
- 3- They show sensitive color reactions of red and violet with ferric perchlorides; therefore one can determine the formation of hydroxamic acids by this and also one can readily detect the completion of analysis or dissolution.
- 4- One can differentiate their solubilities for various solvents by saturated or unsaturated acids or by the number of carbon chains.
- 5- It is convenient for obtaining especially pure isolated fatty acids; it is convenient also for comparing various other derivatives used in determination methods, for example derivatives of FENACHIRU /T.N. possible phenathyl etc./.

The following reaction formulas describe the operations:



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Actually the molecular ratio followed is thus-

ester: $\text{NH}_2\text{OH} \cdot \text{HCl} : \text{Na}$ equals 1: 1.1: 2.1

The fatty acid ethyl ester is dissolved in anhydrous ethanol; to this is added an anhydrous methanol solution of hydroxylamine chlorate salt; into this mixture is allowed to drop an anhydrous ethanol solution of sodium ethylate which was prepared beforehand; then the mixture is set aside for 24 to 28 hours at room temperature.

If especially great care is taken to guard against water then it is felt that the reaction will proceed quantitatively.

The sodium chloride thus formed will for the greater part be neutralized first by a chlorate of ethanol and then in an ethanol solution of glacial acetic acid. It is neutralized until it is only slightly acidic with respect to methyl orange or Congo red; then immediately it is mixed with water and cooled in ice.

Then the isolated hydroxamic acid is caused to crystallize.

Furthermore, in order to recrystallize it in various solutions or in order to purify it further, then an ethanol solution of neutral copper acetate is added to the ethanol solution of hydroxamic acid causing a copper salt to precipitate; after washing it is suspended in ethanol and is analysed with sulfuretted hydrogen and again free hydroxamic acid is obtained.

EXPERIMENTS:

BEEEN-Possible B.N. i.e. Beta Normal hydroxamic Acid

We dissolve 1.5 g of $\text{NH}_2\text{OH} \cdot \text{HCl}$ into 70 cc of anhydrous methanol; add 300 cc of anhydrous ethanol; and then finally we add to the solution 7.3 g of ethyl ester of B.N. acid.

Separately we dissolved 1.1 g of metallic sodium in 100 cc of anhydrous methanol; then we added this to the above-mentioned alcoholic mixture of $\text{NH}_2\text{OH} \cdot \text{HCl}$ and ethyl ester of B.N. acid while stirring the mixture. The white precipitate that is immediately formed is ordinary table salt. By tightly sealing the container we guard against the entrance of moisture; furthermore we prevent the decomposition of the ethyl ester of the B.N. acid; the result is heated to about 30°Centigrade and is kept continuously stirred and then finally set aside.

Slowly a white crystalline precipitate is formed.

We take a small amount of the test material and make it slightly acidic with some glacial acetic acid; when we add a few drops of ethanol solution of ferric perchloride the test material turns a beautiful red-purple color.

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After two days the material is cooled; the precipitate resulting from the cooling is then filtered out; then the precipitate is washed and dried. The filtered liquid shows almost no color reaction with an ethanol solution of ferric perchloride.

The sodium salt of hydroxamic acid is almost insoluble in chilled alcohol.

The dried precipitate is for the most part neutralized in ethanol by ethanol-hydrochloric acid; furthermore it shows slight acidity with Congo red in ethanol glacial acetic acid; it is slightly heated and then quickly chilled; with the addition of cold water complete precipitation is brought about; finally it is filtered, washed with water, and dried.

It cannot be dissolved even if it is washed in ether; the amount obtained is 6.4 g (90%).

It is recrystallized for the second time from anhydrous alcohol; its melting point is 112.5°.

It is soluble when heated in methyl and ethyl alcohols; soluble only with difficulty in acetone; and is insoluble in water, ether, and petroleum ether.

ANALYSIS:

actual weight of test material : 2.914 mg
atmospheric pressure : 752.2 mm
temperature : 16°
nitrogen : 3.955 (0.102 cc)

theoretical formula : $C_{12}H_{25}O_2N$
theoretical value of nitrogen : 3.94%

When a surplus of an ethanol solution of neutral copper acetate is added to the ethanol solution, there is formed a large volume of green precipitate.

(2) Arachic-hydroxamic Acid

The ethyl ester of arachic acid is synthesized from stearic acid in the following steps: That is, stearic acid \rightarrow ethyl ester stearyl alcohol \rightarrow iodized stearyl \rightarrow stearyl malonic acid diethyl ester \rightarrow stearyl malonic acid \rightarrow arachic acid \rightarrow arachic acid ethyl ester.

The Production of Arachic Hydroxamic Acid:

0.3 g of $NH_2OH \cdot HCl$, 1.0 g of arachic acid ethyl ester synthesized as above (melting point 50°), 0.2 g of metallic sodium, 40 cc of methanol and 240 cc of anhydrous ethanol; there is a reaction completely similar to that in the case of B.N. hydroxamic acid; 0.85 g of arachic hydroxamic acid is obtained (92%); melting point is 109°.

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It is recrystallized a second time and the melting point is 109.5 to 110°.

ANALYSIS:

actual weight of test material : 3.793 mg
 atmospheric pressure : 761.5 mm
 temperature : 14.5° C
 nitrogen : 0.139 cc
 percent of nitrogen : 4.22%
 theoretical formula : $C_{20}H_{41}O_2N$
 theoretical percent of nitrogen : 4.28%

For various solvents, this chemical has roughly the solubility as BEEENTO [possibly an error for B.N.] hydroxamic acid.

(3) Stearic Hydroxamic Acid:

Stearic acid ethyl ester 10.4 g in amount, 2.55 g of $NH_2OH.HCl$, 50 cc of anhydrous methanol, 200 cc of anhydrous ethanol, and 1.6 g of metallic sodium; the reaction is similar to that of the above-mentioned Arachic hydroxamic acid; the resultant crystals dissolve in ethyl alcohol; it is cooled by the addition of petroleum ether; when it is recrystallized we obtain 8.7 g (92%) of a large mass of white aculeate crystals whose melting point is 106° to 106.5°. If it is recrystallized a second time from ethanol petroleum ether, then we find its melting point is 106.5° to 107° (according to Morelli and Lewis it is 104°).

ANALYSIS:

actual weight of the test material : 2.252 mg
 amount of carbon dioxide : 5.963 mg
 amount of water : 2.474 mg
 percent of carbon : 72.20%
 percent of hydrogen : 19.29%
 theoretical formula : $C_{18}H_{37}O_2N$
 theoretical percent of carbon : 72.17%
 theoretical percent of hydrogen : 12.46%

actual weight of test material : 3.441 mg
 atmospheric pressure : 752.4 mm
 temperature : 16.00 C
 amount of nitrogen : 0.144 cc (4.72%)
 theoretical formula : $C_{18}H_{37}O_2N$
 theoretical percent of hydrogen : 4.68%

Stearic hydroxamic acid easily dissolves when warmed in alcohol and acetone; it is insoluble in petroleum ether, and water.

(4) palmitic hydroxamic acid :

Ethyl esters of palmitic acid (melting point 24°) in the amount 14.2 g, 4.2 g of $NH_2OH.HCl$, 80 cc of anhydrous methanol, 2.5 g of metallic sodium, all in 220 cc of anhydrous ethanol; the reaction is entirely similar to that of stearic hydroxamic acid:

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in carrying out the operations we obtain 12.6 g (yield 93%) of palmitic hydroxamic acid.
It is recrystallized three times from ethyl alcohol petroleum ether; melting point is 102.5°.

ANALYSIS:

actual values as determined:

weight of the test material	: 4.341 mg
atmospheric pressure	755.1 mm
temperature	15.0°
amount of nitrogen	0.201 cc (5.27%)

theoretical values:

formula	$C_{16}H_{33}O_2N$
amount of nitrogen in percent	5.16

The solubility of palmitic hydroxamic acid in the various solvents is for the most part similar to stearic hydroxamic acid. (According to Morelli and Lewis its melting point is 99° and 100° respectively; see the note at the end.).

(5) Myristic Hydroxamic Acid:

Myristic acid ethyl ester in the amount 10.5 g (melting point is 157°/8mm), 3.3 g of $NH_2OH \cdot HCl$, 60 cc of anhydrous methanol, 2.4 g of metallic sodium, and 140 cc of anhydrous ethanol are caused to react in a manner similar to a reaction with palmitic hydroxamic acid. After two days the reacting solution is neutralized to a great extent with ethanol hydrochloric acid; it is made slightly acidic with respect to Congo red by ethanol-glacial acetic acid and then completely dissolved; the hydroxamic acid resulting by the cooling and the addition of cold water is completely precipitated out in the form of crystals; then it is filtered, washed and dried.
It is dissolved in a small quantity of anhydrous ethanol and heated; cooled by the addition of petroleum ether; recrystallized and filtered out; washed with petroleum ether; and finally 8.6 g of myristic hydroxamic acid whose melting point is 98° is obtained (95%).
This operation is carried out similarly two times and recrystallized; melting point is 98 to 98.5°.

ANALYSIS:

actual values obtained:

weight of the test material	3.120 mg
atmospheric pressure	750.6
temperature	15.5°
amount of nitrogen	0.154 cc (5.57%)

theoretical values:

formula	$C_{14}H_{29}O_2N$
amount of nitrogen in percent	5.76

(6) Lauric Hydroxamic Acid:

(a) $NH_2OH \cdot HCl$ in the amount of 0.7 g, 2.0 g of Lauric acid ethyl

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ester (boiling point 132 to 133°/8mm), 50 cc of anhydrous methanol, 0.43 g of metallic sodium, 150 cc of anhydrous ethanol are caused to react as in the case with Myristic hydroxamic acid.

Even after two days there is little that can be thought of as a precipitate of a sodium salt of hydroxamic acid; the reacting solution shows reaction with ferric perchloride.

The reacting solution is for the most part neutralized by ethanol hydrochloric acid first; then the ordinary table salt that is formed is filtered out; the remaining filtered solution shows slight acidity with respect to Congo red in ethanol-glacial acetic acid; when one adds an excess solution of neutral copper acetate ethanol, then there is formed a large quantity of green amorphous precipitate. The precipitate is filtered out and then washed in dilute ethanol; it is again suspended in ethanol and hydrogen sulfide is passed through it; the copper separates out; and finally copper sulfide formed is filtered out. The filtered solution remaining is then purified of its color by passing through bone charcoal; it is concentrated until crystals appear at low temperature (below 50°) and decreased pressures; furthermore it is cooled by the addition of petroleum ether and is caused to precipitate out as crystals.

Once more it is dissolved in a small quantity of ethanol; crystals are formed by the addition of petroleum ether; the resulting hydroxamic acid has a melting point of 94°; the amount obtained is 1.5 g (85%).

(b) Lauric acid ethyl ester in the amount of 10.0 g $\text{NH}_2\text{OH} \cdot \text{HCl}$ in the amount 3.1 g, 60 cc of anhydrous methanol, 2.0 g of metallic sodium, all in 140 cc of anhydrous ethanol. the reaction is as in case (a) above.

The reacting solution is neutralized in a cool solution of ethanol hydrochloric acid; the ordinary table salt is filtered out.

The filtered solution is concentrated at low temperature and at decreased pressures; furthermore, it is made slightly acidic with respect to Congo red in ethanol-glacial acetic acid.

It is rapidly cooled; precipitated out completely in crystalline form by the addition of cold water; then filtered, washed in water, and dried.

It is again heated and dissolved in a small quantity of ethanol; cooled by the addition of petroleum ether; made to precipitate in crystalline form; then filtered, and washed in petroleum ether.

The quantity obtained is 8.2 g (94%).

Furthermore it is caused to crystallize a second time in ethanol petroleum ether; Lauric hydroxamic acid is obtained whose melting point is 94° C.

(The melting point according to Lewis is 82.5°; see note at the end.)

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ANALYSIS:

actual values as measured:

weight of the test material	3.580 mg
atmospheric pressure	750.4 mm
temperature	15.5°C
amount of nitrogen	0.205 cc (6.46%)

theoretical values:

formula	$C_{12}H_{25}O_2N$
amount of nitrogen in percent	6.51

Lauric hydroxamic acid is quite soluble in ethanol, methanol and acetone; it is insoluble in petroleum ether and water; and it is soluble in ether.

(7) Capric Hydroxamic Acid:

(a) Capric acid ethyl ester (boiling point is 244 to 545° C) in the amount 2.5 grams, $NH_2OH \cdot HCl$ in the amount of 0.9 gram, 50 cc of anhydrous methanol, metallic sodium in the amount of 0.5 gram, all in 150 cc of anhydrous ethanol; from this reaction is obtained 2 grams (85%) of capric hydroxamic acid of boiling point 88 to 88.5° C, as in the above reaction (a) with Lauric hydroxamic acid.

(b) Capric acid ethyl ester in the amount of 10.0 grams, $NH_2OH \cdot HCl$ in the amount of 3.9 grams, 80 cc of anhydrous methanol, 2.5 grams of metallic sodium, all in 140 cc of ethanol; the reaction is similar to that of (b) with Lauric hydroxamic acid; properly treated the solution yields capric hydroxamic acid.

The amount obtained is 8.4 grams (90%).

The washed filtered solution presents a red color in a solution of ferric perchloride; it will dissolve in a considerable quantity of water.

It is crystallized a second time from anhydrous ethanol petroleum ether; the resultant has a melting point of 88 to 88.5° C.

Capric hydroxamic acid is readily soluble in alcohol and acetone; it is soluble only with difficult in ether; it is insoluble in petroleum ether;

ANALYSIS:

actual values as obtained for the test material;

weight	2.736 mg
amount of carbon dioxide	6.405 mg
amount of water	2.790 mg
percent of carbon	63.83 %
percent of hydrogen	11.41 %

theoretical values:

formula	$C_{10}H_{21}O_2N$
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percent of hydrogen	64.11%
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actual values as obtained for the test material:

weight	2.845 mg
atmospheric pressure	751.8 mm
temperature	16.0° C
amount of nitrogen	0.195 cc (7.73%)

theoretical values:

weight	11.31 mg
amount of nitrogen	7.48 %

(8) Caprylic Hydroxamic Acid:

(a) Ethyl ester of caprylic acid in the amount of 3.4 grams (boiling point 206 to 207°), 1.6 gram of $\text{NH}_2\text{OH}\cdot\text{HCl}$, 50 cc of anhydrous methanol, 1 gram of metallic sodium, and 150 cc of anhydrous ethanol; the reaction is similar to that of (a) with capric hydroxamic acid; then it is treated; a copper salt is released; finally 2.6 grams of caprylic hydroxamic acid is obtained whose melting is 78.5 to 79° C. The amount obtained is 80%.

(b) Caprylic acid ethyl ester in the amount of 9.3 grams, 4.5 grams of $\text{NH}_2\text{OH}\cdot\text{HCl}$, 80 cc of anhydrous methanol, 2.8 grams of metallic sodium, and 140 cc of anhydrous ethanol; the reaction is similar to that above.

The reacting solution shows no precipitate at all except for the precipitation of common ordinary table salt.

The sodium salt of the caprylic hydroxamic acid which is produced is completely dissolved. After it is treated in a manner similar to that in case (b) with capric hydroxamic acid, 7.6 grams of caprylic hydroxamic acid is obtained. (89%).

It is recrystallized a second time from ethanol, petroleum, ether or ethyl alcohol ether; the melting point of the crystal is 78.5 to 79° C.

ANALYSIS:

actual values:

weight	3.179 mg
air pressure	755.3 mm
temperature	15.0° C
nitrogen	0.249 cc (8.92%)

theoretical values:

formula	$\text{C}_8\text{H}_{17}\text{O}_2\text{N}$
nitrogen	8.80 %

Caprylic hydroxamic acid dissolves readily in alcohol and acetone; it can dissolve to a considerable degree in ether and water; and will not dissolve in petroleum ether at all.

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(9) Capronic ~~Caproic~~ Hydroxamic Acid:

(a) Ethyl ester of capronic acid (melting point is 164 to 165°C) in the amount of 4.5 grams, $\text{NH}_2\text{OH}\cdot\text{HCl}$ in the amount 2.4 grams, 70 cc of anhydrous methanol, 1.5 grams of metallic sodium, and 130 cc of anhydrous ethanol; the reaction is as in the previous one.

The reacting solution releases a copper salt as in (a) with caprylic hydroxamic acid; 3.4 grams of capronic hydroxamic acid is finally obtained (83%).

(b) Ethyl ester of capronic acid in the amount of 14.4 grams, 7.7 grams of $\text{NH}_2\text{OH}\cdot\text{HCl}$, 100 cc of anhydrous methanol, 4.9 grams of metallic sodium, and 200 cc of anhydrous methanol; the reaction is similar to the above.

The reacting solution shows no precipitate except common table salt. Two days later it is cooled and neutralized for the most part in cold ethanol hydrochloric acid; the solvent is almost all distilled away at comparatively low temperatures and decreased pressures; then a small quantity of water is added; it is cooled; made slightly acidic with respect to Congo red in acetic acid; finally extracted several times in ether.

The ether extracting solution is dried with anhydrous sulfate of soda and then the great part of the ether is distilled away; then it is cooled by the addition of petroleum ether; finally the hydroxamic acid precipitates out in a crystalline form. It is filtered and washed in petroleum ether.

The quantity obtained is 11.2 grams (85%).

In both (a) and (b), the materials are recrystallized twice in ether and petroleum ether. The melting points are 63.5 to 64°C.

ANALYSIS:

actual values:

weight of the test material	3.654 mg
atmospheric pressure	796.1 mm
temperature	14.5°C
amount of nitrogen	10.50% (0.335 cc)

theoretical values:

formula	$\text{C}_6\text{H}_{13}\text{O}_2\text{N}$
amount of nitrogen	10.68%

Capronic hydroxamic acid is readily soluble in acetone, alcohol, and ether; it is soluble somewhat in water; it is not soluble in petroleum ether.

(10) Butyl Hydroxamic Acid:

(a) Ethyl ester of butyric acid in the amount 2.8 grams (its boiling point is 198 to 199°C), 1.7 gram of $\text{NH}_2\text{OH}\cdot\text{HCl}$, 50 cc of anhydrous methanol, 1.1 gram of metallic sodium, and 160 grams of anhydrous ethanol; the reaction is as previously described.

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Copper salt, as in the case (a) with capronic hydroxamic acid, is released; the ethanol is distilled away at comparatively low temperatures and at decreased pressures; we finally obtain a sticky brown-colored substance.

The color is removed with difficulty by bone charcoal and finally the color becomes a pale yellow.

When it is cooled by a refrigerant it becomes semi-hard body; however, it does not crystallize.

It dissolves in a small quantity of acetone or ethyl alcohol; if ether or petroleum ether is added the second precipitate does not crystallize even after repeated attempts.

(b) Ethyl ester of butyric acid in the amount of 5.9 grams, 3.8 grams of $\text{NH}_2\text{OH} \cdot \text{HCl}$, 70 cc of anhydrous methanol, 2.4 grams of metallic sodium, and 230 cc of anhydrous ethanol; the reaction is similar to the others above.

Even after three days there is no precipitate except common table salt.

Similarly it is neutralized by the chloride of ethanol; it is slightly acidic with respect to Congo red in ethanol glacial acetic acid; and at comparatively low temperatures and pressures the solvent is distilled away.

That which precipitates out during the distilling (chiefly ordinary table salt) is filtered out; when the solvent is almost evaporated a substance of a light yellow color remains. It dissolves in a small quantity of anhydrous ethanol; when petroleum ether is added a semi-liquid substance of pale yellow color precipitates just as in the case (a) above. We carried out repeated precipitation from ethanol petroleum ether; even when cooled no crystals formed.

With ferric perchloride, it possesses a characteristic color reaction of hydroxamic acid. Furthermore with a solution of copper butyrate it forms a large mass of amorphous green precipitate; thus is formed hydroxamic acid.

If there is no water during neutralization, it will not be completely unstable to the extent of decomposing, as was suggested by Lewis.

It is readily soluble in alcohol; it is soluble somewhat in water and in acetone; it is soluble only with difficulty in ether; and it is not soluble at all in petroleum.

(11) Propionic Hydroxamic Acid:

Propionic ethyl ester (boiling point 99°) in the amount of 5.1 grams, 3.8 grams of $\text{NH}_2\text{OH} \cdot \text{HCl}$, 70 cc of anhydrous methanol, 2.4 grams of metallic sodium, 230 cc of anhydrous ethanol; the reaction is as previously carried out; propionic hydroxamic acid is obtained in this reaction.

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It is recrystallized three times from ethanol and acetone; the crystal has a melting point of 92.5 to 93° C (according to A. Miolati it is 85°; see the end of the document)

ANALYSIS :

actual values:

weight of the test material	3.974 mg
atmospheric pressure	757.2 mm
temperature	12.5° C
amount of nitrogen	0.543 cc (15.77%)

theoretical values:

formula	$C_3H_7O_2N$
amount of nitrogen	15.73%

(12) Acetohydroxamic Acid:

Ethyl ester of butyric acid (boiling point 77°C) in the amount of 8.0 grams, 7.6 grams of NH_2OH , HCl. 130 cc of anhydrous methanol, 6.4 gram of metallic sodium, and 270 cc of anhydrous ethanol; the reaction is similar to the previous ones; acetohydroxamic acid is obtained.

That which is caused to precipitate by the addition of petroleum ether to the ethanol is a semi-liquid substance; however when it is cooled it hardens and turns to a crystal. It is recrystallized from ethyl alcohol and acetone; dried in a vacuum sulfuric acid desiccator for two days; when the water of crystallization is driven off the material then possesses a melting point of 88°C (according to Hoffman it is 87 to 88°; see the end of this document).

ANALYSIS:

actual values:

weight of the material	2.666 mg
atmospheric pressure	762.5 mm
temperature	14.0°C
amount of nitrogen	0.425 cc (18.44%)

theoretical values:

formula	$C_2H_5O_2N$
amount of nitrogen	18.66%

These twelve hydroxamic acids described above all clearly show a violet and red color reaction with ferric perchloride in a solution of ethanol.

Furthermore they all form, with neutral acetic acid in solution with ethanol a large mass of amorphous copper salt precipitate colored green.

Also they all reduce to the original hydroxamic acids by means of sulfuretted hydrogen.

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Hydroxamic Acids

Fatty Acids	Melting Point of the Original Fatty Acids	Melting Point	Ethyl Alcohol	Acetone	Ether	Water	Petroleum Ether
C22	84	112.5	++	+	-	-	-
C20	77	109.5-110	++	+	-	-	-
C18	71.5-72	106.5-107	+++	++	-	-	-
C16	63.5-64	102.5	+++	++	-	-	-
C14	57.5-58	98-98.5	++++	+++	+	-	-
C12	47.5-48	94	++++	+++	++	-	-
C10	31.5	88-88.5	+++++	++++	+++	+	-
C8	16	78.5-79	+++++	++++	++++	++	-
C6	-1.5	63.5 - 64	+++++	++++	++++	+++	-
C4	-4.7	vicid liquid	+++++	+++	++	+++	-
C3	-19.7	92.5-93	+++++	+++	+	+++++	-
C2	16.7	88	+++++	+++	+	+++++	-

(- means insoluble; + means soluble. The number of pluses or minuses indicates the degree of solubility or insolubility.)

The table above shows the differences in the solubilities of the various hydroxamic acids in ethyl alcohol, acetone, ether, petroleum ether and water.

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NOTE: The fatty acids used in these experiments were prepared in accordance with the standards of Merck and Kahlbaum or Theodore Schuchardt the acids were esterized and then purified by recrystallization or distillation.

However the ethyl ester of butyric acid was prepared in pure form by repeated distillation of ordinary market materials as sold in the city.

We wish to express our gratitude to the Japanese Technical Advancement Society (NIPPON GAKUJUTSU SHINKOKAI), who assisted in part by paying for the expense of these experiments.

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END

PART TWO: UNSATURATED HYDROXAMIC ACIDS

Concerning unsaturated fatty acids, it is possible to synthesize many various derivative compounds by reactions relating to the carboxyl radical and the unsaturation bond between molecules of aliphatic acids.

Thus one is lead to crystal derivative compounds which possess definite and accurate physical constants, from unsaturated fatty acids.

Therefore one can facilitate the chemical separation and determination of these acids; this is especially important from the standpoint of chemical studies on fatty acids.

The authors first prepared hydroxamic acids from saturated fatty acids which possessed an even number of carbon atoms from C2 to C22; then determined the melting points of the various hydroxamic acids.

Here in the following work we follow much the same method we used previously in the preparation of hydroxamic acids of saturated fatty acids of the first report; we prepared the various hydroxamic acids of the three unsaturated fatty acids, oleic, linolic, and linolenic acid; their respective melting points were determined to be 61°, 41° to 42° and 37° to 38°.

They all possessed a color reaction with ferric perchloride in an alcohol solution; the color was a beautiful violet and red; moreover in neutral ethanol solution of copper acetate there was formed a large mass of an amorphous precipitate which was green and insoluble.

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All these facts are completely similar to those in the case of hydroxamic acids of saturated fatty acids.

In this method is thought that the hydroxylamine is not active at the point of the double bond.

The oleic, linolic, and linolenic Hydroxamic acids are all insoluble in water. They all dissolve readily in the various organic solvents such as alcohol, ether, acetone, benzol, petroleum ether, chloroform, and carbon tetrachloride.

The degree of their solubilities is as follows:

oleic is less than linolic and linolic is in turn less than linolenic hydroxamic acid.

The sodium salts of these acids are all readily soluble in alcohol. When one compares these facts and the characteristics of hydroxamic acids of the saturated acid series described in the first part, it can be thought that both the saturated fatty acid series and the hydroxamic acids of the unsaturated fatty acid series are analyzable.

Concerning the hydroxamic acids of the unsaturated acid series, one can refer to the old reports of Morelli and Lewis (see the note at the end) on the oleic hydroxamic acid (melting point 61°).

THE EXPERIMENT :

Oleic, linolic, and linolenic hydroxamic acids are produced by the reaction of hydroxylamine on sodium ethylate in an alcohol solvent with the ethyl ester; this reaction is similar to that in the case of the production of the hydroxamic acid of the saturated fatty acids.

That is to say, 1.1 mole of hydroxylamine hydrochloride salt is dissolved in anhydrous methanol; then anhydrous ethanol is added; at the same time one mole of ethyl ester of the fatty acid is dissolved,

Next we dissolve 2.1 moles of metallic sodium into anhydrous ethanol; then the above-mentioned is added to an alcohol solvent containing the ethyl ester of the fatty acid in question and hydroxylamine hydrochloride salt; the whole solution is agitated well and finally is set aside for 36 hours at room temperature. All this time the solution is kept stirred well.

Upon completion of the reaction the solution is cooled; first it is neutralized for the most part in alcohol hydrochloric acid or in alcohol glacial acetic acid (it is alkaline with respect to Congo red); then the greater part of the solvent is distilled away under reduced pressure and at as low a temperature as possible; and finally while it is being cooled it is made slightly acidic with respect to Congo red in ethanol glacial acetic acid; and then when mixed with a large quantity of cold water a crystal white precipitate is yielded.

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The precipitate is filtered; after it is washed in water it recrystallized from petroleum ether, ether petroleum ether, or alcohol petroleum ether.

Otherwise, we add a saturated solution of alcohol of neutral copper acetate to the test material slightly acidified as above with respect to Congo red; the hydroxamic acid thus formed is caused to precipitate out as a copper salt; this precipitate is separated; then it is placed in alcohol; it is reacted upon by sulfuretted hydrogen; then the solvent from which the copper sulfide is filtered is distilled away at reduced pressure and low temperature; finally the remaining sludge can be prepared in pure form by recrystallization as in the methods described above.

Oleic Hydroxamic Acid:

Ethyl ester of oleic acid is prepared in the standard way from oleic acid (Merck; iodine number 85.66); the product resulting from esterizing it alcohol hydrochloric is distilled twice under reduced pressure (boiling point 209 to 211°/7mm).

From 10 grams of this ethyl ester is obtained 9 grams of hydroxamic acid; it is recrystallized four times from petroleum ether; finally we obtain 7.3 grams of oleic hydroxamic acid of boiling point 61°.

ANALYSIS:

test material weight	1.945 mg
carbon dioxide	5.176 mg
water	2.064 mg
carbon	72.57%
hydrogen	11.87%
theoretical formula	$C_{18}H_{35}O_2N$
theor. am't of C	72.69 %
theor. am't of H	11.85 %

test material weight	3.080 mg
air pressure	763.0 mm
temperature	17.5°C
nitrogen	0.125 cc (4.62%)
theoretical formula	$C_{18}H_{35}O_2N$
theor. am't of N	4.71 %

Linolic Hydroxamic Acid:

We select a mixed fatty acid by saponifying by the usual standard methods soy bean oil (saponification number 191.6, iodine number 140.99); it is bromidized and thus tetrabromide stearate is obtained (melting point 114°); then it is esterized with zinc powder and ethanol sulfuric acid simultaneously with debromidization.

Hydroxamic acid is prepared from 10.5 grams of the ethyl ester of linolic acid thus obtained, in the same manner as described above (the iodine number of linolic acid ethyl ester is 159.82)

It is extracted three times from petroleum ether; there results 6.8 grams of linolic hydroxamic acid of melting point 41 to 42°.

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The material is white squamate crystals.

Its sodium salt dissolves readily in alcohol.

ANALYSIS:

test material weight	2.049 mg
carbon dioxide	5.510 mg
water	2.060 mg
carbon	73.33 %
hydrogen	11.24 %

theoretical formula	$C_{18}H_{33}O_2N$
theor. am't of C	73.22%
theor. am't of H	11.18%

test material weight	2.329 mg
air pressure	763.1 mm
temperature	17.5 °C
theoretical formula	$C_{18}H_{33}O_2N$
theor. am't of H	4.74%

Linolenic Hydroxamic Acid:

As in the operations above, we treat linseed oil (saponification number 189.86, iodine number 178.3); that is, by the usual standard methods we saponify the oil and thus prepare a fatty acid compound; by bromidization we obtain hexabromide stearate (melting point 182 to 183°); finally it is esterized with zinc powder and ethanol sulfuric acid simultaneously with debromidization).

The 12.5 grams of linolenic acid ethyl ester thus obtained (iodine number 234.54) is caused to produce a hydroxamic acid by a reaction similar to those above.

It is extracted three times from petroleum ether and thus we finally obtain 6 grams of linolenic hydroxamic acid of melting point 37 to 38°.

It is readily soluble at room temperature even in a small quantity of petroleum ether; it also dissolves so readily in other solvents even at 0°C that it can hardly be recrystallized from them.

ANALYSIS:

test material weight	2.935 mg
carbon dioxide	7.942 mg
water	2.801 mg
carbon	73.79 %
hydrogen	10.67 %

theoretical formula	$C_{18}H_{31}O_2N$
theor. am't of C	73.66 %
theor. am't of H	10.65 %

test material weight	4.435 mg
air pressure	762.8 mm
temperature	17.5 °C

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nitrogen	0.191 cc (4.90%)
theoretical formula	$C_{18}H_{31}O_2N$
theor. am't of N	4.77%

The ethanol solutions of all the above-mentioned four acids show a beautiful red and violet color reaction readily with ferric chloride furthermore, they are green in an ethanol solution of copper acetate.

An amorphous insoluble precipitate is formed.

Oleic hydroxamic acid can be recrystallized from alcohol and even ether; also it is possible to recrystallize linolic hydroxamic acid from ether.

We wish to express our sincere thanks to the Japanese Technical Advancement Society for helping us to defray part of the costs of these experiments.

END

[End of DOCUMENTS BRANCH TRANSLATION No 63]

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